Managing biological variation in skin background colour along the postharvest chain of Jonagold apples

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Abstract

Skin background colour is an important quality aspect in the grading of Jonagold apples, with consumers usually preferring fruit with a green background colour. However, apple handlers are usually faced with large fruit-to-fruit variability of background colour within a population of fruit. In this study, a stochastic modelling approach was used to describe how the initial fruit-to-fruit variability in the background colour of Jonagold apples present at harvest, propagates throughout the postharvest chain. Two hundred and twenty Jonagold apple fruit were harvested and stored at 1 ºC or 4 ºC, under different controlled atmosphere (CA) conditions for six months, followed by two weeks exposure to shelf life conditions, during which the background colour and ethylene production of the individual fruit were measured. A kinetic model was developed to describe the postharvest loss of skin greenness, by assuming that the loss was principally due to chlorophyll breakdown, the rate of which was dependent on the endogenous ethylene concentration. Stochastic model parameters were identified, and by treating these parameters as fruit-specific, the model could account for more than 95 % of the variability of the data. By treating the stochastic model parameters as random factors, the Monte Carlo method was used to model and describe the propagation of the fruit-to-fruit variability of the background colour within a population of fruit. The model developed in this study can allow better

management of variability in quality along the postharvest chain, by predicting how the initial fruit-to-fruit variability within a batch of apple will propagate throughout the postharvest chain, as a function of storage and shelf life conditions.

Keywords: Malus × *domestica*, Kinetic modelling, Monte Carlo, Ethylene production, Fruit quality

1. Introduction

Once harvested, fresh fruits and vegetables undergo continuous degradation in quality due to various oxidative processes, such as respiration and ethylene production, occurring within the cells. Postharvest handlers attempt to limit these reactions implicated in quality breakdown by storing fresh fruits and vegetables at low temperature in an atmosphere with reduced O_2 concentration and elevated CO_2 concentrations, hence prolonging their storage life (Kader 1986). Jonagold apple, the commercially most important apple cultivar in Belgium, is characterized by red skin colouration, with a green background colour. During storage, the green skin background colour of Jonagold apples turns yellow as the chlorophyll pigments within the skin are broken down to colourless products, and in the process the underlying yellow pigments are unmasked (Müller et al., 2007; Kräutler 2008).

Biological variation is inherent in biological produces. For example, even fruit harvested on the same day from the same tree, may still show large variation in maturity due to differences in orientations on the tree, flowering dates, level and type of fungicides, shades etc. Therefore, individual Jonagold apples within a batch of fruit (fruit of the same cultivar harvested from one grower on the same day) show large variability in their skin background colour at harvest, and this propagates throughout the postharvest chain. The greenness of the background colour of the skin is an important quality indicator for grading of Jonagold apples. Apples with extremely green background colour are graded in the top quality classes, and have higher commercial values. Therefore, understanding how the initial fruit-to-fruit variability in skin background colour propagates throughout the postharvest chain will enable fruit handlers to predict what proportion of the fruit will belong to certain quality classes at the end of storage, and how long they can stay under shelf conditions before a given proportion gets beyond consumer acceptance.

Kinetic modelling in the postharvest sciences provides a useful tool to integrate existing knowledge on mechanisms underlying biological processes and new experimental data. It enables postharvest handlers to be able to predict what-if scenarios, and could be used in optimizing food handling in the postharvest chain. Since biological variation is inherent in biological produces, it is important to account for biological variation when developing models for the postharvest sciences (Tijskens et al., 2003). Several techniques have been used to model variability in quality along the postharvest chain (Nicolaï at al., 1999; Hertog, 2002; Schouten et al., 2002; Hertog et al., 2004; Scheerlinck et al., 2004; Hertog et al., 2007a; Tijskens et al., 2008; Mziou et al., 2009; Gwanpua et al., 2013; Jordan and Loeffen, 2013). Most of these models, though having a sound conceptual base, do not incorporate the regulatory role of endogenous ethylene. It is well established that ethylene plays a key role in regulating various quality breakdown reactions during ripening (Lidster et al., 1983; Saltveit, 1999; Stow et al., 2000; Watkins et al., 2000; Johnston et al., 2009; Tacken et al., 2010), but very little research has been done to model the effect of ethylene on quality breakdown. Van der Sman and Sanders, (2012) developed a biological switch model to describe how ethylene triggers softening in apples. Also, the effect of ethylene on apple softening has been modelled by Gwanpua et al., (2012). However, such models linking ethylene to quality losses have not been extended to colour breakdown (and/or synthesis). Hertog et al. (2004) used an approach, initially developed by Tijskens and Evelo (1994), to model the postharvest behaviour of colour in tomatoes, in which they assumed that the change in colour during ripening is due to enzymatic chlorophyll degradation, and that these enzymes increase exponentially due to autocatalytic ethylene production. However, no data on ethylene production was included in that study. Schouten et al. (2002) developed a model for chlorophyll breakdown and synthesis during storage of cucumber, but the regulatory effect of endogenous ethylene was not accounted for.

The objective of this study was to provide practical guidelines for managing biological variation in skin background colour of apples along the postharvest chain, by using a stochastic kinetic modelling approach. We aimed at developing a model that incorporates the main physiological processes involved in postharvest chlorophyll loss, including the regulatory role of endogenous ethylene, to

explain the loss of skin greenness in apples during storage at different conditions, and subsequent exposure to shelf life conditions. Furthermore, the Monte Carlo method was used to describe the propagation of the fruit-to-fruit biological variability in green background colour of Jonagold apples during postharvest handling.

2. Materials and methods

2.1. Fruit

Jonagold apples (*Malus* × *domestica* Borkh.) were harvested from the Van der Velpen Company located at Opvelpsestraat, Bierbeek, Belgium. Apples were picked on 22nd September 2012, which was within the window for the optimal picking dates for Jonagold apples in that season, and transported immediately to the Flanders Centre of Postharvest Technology (VCBT), Belgium, where the storage experiments were done. The optimal picking dates were determined by the VCBT, based a combination of measurements of firmness, starch index, soluble solids content, colour and respiration rates. 220 fruit were randomly selected from the batch of apples and numbered, and a circular region with a diameter of about 2 cm was marked on the green skin for each fruit.

2.2. Storage experiments

The apples were randomly divided into groups of 20 and each group was stored at one of the following 10 storage conditions: 1 kPa O_2 with 3 kPa CO_2, 1 kPa $O_2$with 10 kPa CO_2, 3 kPa O_2 with 3 kPa CO_2, 3 kPa O_2 with 10 kPa CO_2, and normal air at either 1 °C or 4 °C, for a duration of six months. The green background colour of the apple skin and ethylene production were measured every two months during storage. At the end of the storage, the apples were placed in shelf life conditions (20.8 kPa O_2, 0.03 kPa CO_2 and 18 °C) for 15 days, during which similar measurements were done every 5 days. One group of the apples were immediately placed in shelf life conditions, without any prior storage.

2.3. Measurements

103 *2.3.1. Colour*

104 Colour was measured as the a^*-value of the CIE colour system using a hand-held spectrophotometer
105 (Model CM-2600D, Minolta, Kontich, Belgium) calibrated to a white tile. The initial value of the
106 colour was taken on the marked circular spot for all 220 fruit and during each sampling time, the
107 colour measurements were done on these same spots.

108 *2.3.2. Chlorophyll extraction and estimation*

109 The green background colour and chlorophyll content of 20 fruit with varying degree of greenness
110 were measured. Colour was measured as described earlier, and the chlorophyll pigment was
111 quantitatively determined from the peel from the same surface on which the colour measurements
112 were taken, using the method developed by Knee (1972). Five disks with diameters of about 0.96 cm
113 were cut from apple peel, freed from underlying pulp, and crushed in liquid nitrogen. The crushed
114 sample was then transferred to 15 mL falcon tubes, and 100 mg of $CaCO_3$ was added to prevent
115 phytinization. 8 mL of a 3:1 (v/v) Chloroform: methanol solution was added, followed by 2 mL of
116 distilled water. The mixture was centrifuged at 4000 rpm for 1 hour, at 4 °C, and the chlorophyll *a*
117 and chlorophyll *b* were determined from the chloroform phase of the extract, using absorption
118 coefficients reported by Wellburn (1994).

119 *2.3.3. Ethylene production*

120 Ethylene production was measured following the protocol described in Bulens et al. (2011). An apple
121 was initially enclosed in a separate jar of 1.7 L and flushed with humidified gas having the same
122 composition and temperature as the atmosphere under which the apple was stored. The flushing was
123 done at a flow rate of 10 L h^{-1}, for a period of 3 h, to allow steady state to be attained between the
124 headspace and the internal atmosphere of the apple. The inlet and outlet of the jars were then closed
125 and 3 mL samples were withdrawn from the jars and analysed by injecting into a CompactGC
126 (Interscience, Louvain-la-Neuve, Belgium) gas chromatograph. Calibration was done by ethylene
127 standards ranging from 50 ppb to 50 ppm. The temperature, the free volume of the jar and the

pressure inside the jar before sampling, were used to convert ethylene concentration (ppm) to nmol by using the ideal gas law. The ethylene production (nmol kg^{-1} s^{-1}) was obtained by doing a second measurement after a period of about 18 h. For ethylene production measurements done at shelf life conditions, the time interval between the first and the second measurements was 3 h because of the much higher rate of ethylene production within the fruit. The ethylene production for all 220 fruit were measured immediately after harvest and during each sampling time.

3. Model development

3.1. Kinetic basis for postharvest loss in skin green background colour

Loss in green background colour was assumed to be a consequence of the breakdown in chlorophyll pigments in the apple skin, by the action of various chlorophyll degrading enzymes. Although many reactions are involved during the complete breakdown of chlorophyll in plant cells, with many different enzymes being implicated (Matile et al., 1996; Hörtensteiner and Kräutler, 2011), it has been suggested that the first reaction catalysed by the enzyme chlorophyllase is the rate-limiting reaction in the loss of the green colour (Harpaz-Saad et al., 2007; Azoulay Shemer et al., 2008). Kräutler (2008) suggested that coloured intermediates during chlorophyll breakdown occur only transiently. For modelling purpose, it was therefore assumed that the loss of skin greenness in apples is due to the breakdown of chlorophyll, Chl, principally via the action of chlorophyllase, Chlase, into colourless non fluorescent chlorophyll catabolites, NCCs, with a rate constant k_{Chl} (Eq. 1).

$$\mathrm{Chl} + \mathrm{Chlase} \xrightarrow{k_{\mathrm{Chl}}} \rightarrow\rightarrow\rightarrow \mathrm{NCCs} + \mathrm{Chlase} \qquad (1)$$

Ethylene plays a key role in loss of skin greenness during fruit ripening by regulating the activities of the enzymes Chlase (Trebitsh et al., 1993; Jakob-Wilk et al., 1999; Hershkovitz et al., 2005). This process was represented by a simple reaction in which ethylene, C_2H_4, acts on precursors for chlorophyllase synthesis, $\mathrm{Chlase}_{\mathrm{pre}}$, to produce the active form of the enzyme, Chlase, with a rate constant k_{Chlase} (Eq. 2).

152 $$\mathrm{Chlase_{pre}} + \mathrm{C_2H_4} \xrightarrow{k_{\mathrm{Chlase}}} \mathrm{Chlase} + \mathrm{C_2H_4} \quad (2)$$

153 Purvis & Barmore (1981) showed that continuous supply of ethylene is needed to sustain the Chl
154 degrading system in calamondin and Robinson tangerine fruits, therefore it was assumed that Chlase
155 is subject to normal protein turnover (Eq. 3).

156 $$\mathrm{Chlase} \xrightarrow{k_{\mathrm{Chlase,deg}}} \mathrm{Chlase_{deg}} \quad (3)$$

157 *3.2. Model equations*

158 From Eqs. (1) – (3), the following sets of ordinary differential equations were derived by assuming
159 first order kinetics and assuming that ,within the apple tissue , $\mathrm{Chlase_{pre}}$ is always present in excess and
160 therefore is not rate limiting (Eqs. (4) – (5)).

161 $$\frac{d[\mathrm{Chl}]}{dt} = -k_{\mathrm{Chl}}[\mathrm{Chl}][\mathrm{Chlase}], \text{ with } [\mathrm{Chl}](t=0) = [\mathrm{Chl}]_0 \quad (4)$$

162 $$\frac{d[\mathrm{Chlase}]}{dt} = k_{\mathrm{Chlase}}[\mathrm{C_2H_4}] - k_{\mathrm{Chlase,deg}}[\mathrm{Chlase}], \text{ with } [\mathrm{Chlase}](t=0) = [\mathrm{Chlase}]_0 \quad (5)$$

163 where $[\mathrm{Chl}]$ (nmol $\mathrm{cm^{-2}}$) is the chlorophyll content of the skin; $[\mathrm{Chlase}]$ (mmol $\mathrm{m^{-3}}$) is the Chlase
164 concentration, $[\mathrm{C_2H_4}]$ (mmol $\mathrm{m^{-3}}$) is the endogenous ethylene concentration; k_{Chl} ($\mathrm{m^3\ mmol^{-1}\ d^{-1}}$),
165 k_{Chlase} ($\mathrm{d^{-1}}$) and $k_{\mathrm{Chlase,deg}}$ ($\mathrm{d^{-1}}$) are the rate constants for chlorophyll breakdown, synthesis of Chlase and
166 the turnover of Chlase, respectively.

167 The ethylene production depends both on the concentration of the respiratory gases, O_2 and CO_2,
168 within the storage atmosphere and on the climacteric stage of the fruit (for climacteric fruits).
169 Furthermore, there is a transition from the preclimacteric stage to the climacteric stage during ripening
170 in climacteric fruit, and this is responsible for the autocatalytic behaviour of ethylene production.
171 Bulens (2012) developed a detailed kinetic model of ethylene production that includes the most
172 important metabolites of the Yang cycle, and incorporates the transition in climacteric state and the

effect of temperature and concentrations of the respiratory gases. Since our objective in the current study was to use ethylene production to accurately predict loss in skin greenness, and not to provide an insight into the physiology and biochemistry of the ethylene biosynthesis, we did not measure metabolites of the Yang cycle. Therefore, we could not use the model of Bulens (2012). However, we modified the ethylene production model developed by Gwanpua et al. (2012), which is based on a simple Michaelis-Menten kinetic, to incorporate the effect of the transition in the climacteric state of the fruit, by assuming that rate of ethylene production is proportional to the change in the climacteric state of the fruit (Eq. (6)).

$$\frac{d\left[\mathrm{C_2H_4}\right]}{dt} = \frac{df_{clim}}{dt}\frac{V_{max,\mathrm{C_2H_4}} P_{\mathrm{O_2}}}{K_{m,\mathrm{O_2},\mathrm{C_2H_4}} + P_{\mathrm{O_2}}\left(1+\frac{P_{\mathrm{CO_2}}}{K_{mu,\mathrm{CO_2},\mathrm{C_2H_4}}}\right)} - k_{diff}\left[\mathrm{C_2H_4}\right],\ \text{with}\left[\mathrm{C_2H_4}\right](t=0) = \left[\mathrm{C_2H_4}\right]_0 \qquad (6)$$

where $\frac{df_{clim}}{dt}$ is the rate of change in the climacteric state of the fruit, $V_{max,\mathrm{C_2H_4}}$ (mmol m^{-3} d^{-1}) is the maximum rate of ethylene production; $K_{m,\mathrm{O_2},\mathrm{C_2H_4}}$ (kPa) and $K_{mu,\mathrm{CO_2},\mathrm{C_2H_4}}$ (kPa) are the Michaelis-Menten constants for ethylene production and uncompetitive inhibition of ethylene production by carbon dioxide respectively; $P_{\mathrm{O_2}}$ (kPa) and $P_{\mathrm{CO_2}}$ (kPa) are the external partial pressure of oxygen and carbon dioxide respectively; k_{diff} (d^{-1}) is the rate of ethylene diffusing from the apple tissue to its surrounding according to Fick's law. For the latter it is assumed that the external ethylene concentration is negligible.

The change in climacteric stage was modelled by a simple logistic switch function (Eq. (7)), similar to the work of Bulens (2012).

$$\frac{df_{clim}}{dt} = k_{clim} f_{clim}(1 - f_{clim}),\ \text{with}\ f_{clim}(t=0) = f_{clim,0} \qquad (7)$$

where f_{clim} is the transition from the preclimacteric stage ($f_{clim} \approx 0$) to the climacteric stage ($f_{clim} \approx 1$) and k_{clim} is the rate at which the transition occurs. Once climacteric maximum is reach, $\frac{df_{clim}}{dt}$ becomes zero, and the ethylene production is switched off. However, endogenous ethylene that was accumulated during production, continues to diffuse out of the fruit (the last term of Eq. (6)), which explains the decrease in ethylene during late ripening.

All rate constants were assumed to be related to temperature according to the Arrhenius model Eq. (8).

$$k_i = k_{i,ref} \exp\left(\frac{E_{a,i}}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right) \quad (8)$$

where k_i is a rate constant, having $k_{i,ref}$ as a reference value at a certain reference temperature T_{ref} (283.15 K); $E_{a,i}$ (J mol^{-1}) is the activation energy for the respective reactions; R (8.3144 J mol^{-1} K^{-1}) is the universal gas constant, and T (K) is the temperature.

3.3. Model outputs

The model outputs were the measured a^*-value of the CIE colour system and the ethylene production. Preliminary data analysis showed that the relationship between chlorophyll and skin colour (a^*) was not linear. The following empirical equation was used to relate chlorophyll content to colour measurements (a^*):

$$a^* = a^*_{\text{C}} - \alpha\left[Chl\right]^{\gamma}, \text{with } a(t=0) = a_0 \quad (9)$$

where a^*_{C} is a fixed part of the background skin colour, related to the yellow pigments that are present in the skin but are not subjected to significant postharvest degradation (Merzlyak & Solovchenko, 2002), α and γ are constants that were estimated by fitting Eq (9) to the measured data on skin chlorophyll content and background colour (a*).

The ethylene production, $\phi_{C_2H_4}$, is related to the internal ethylene concentration by the following expression:

$$\phi_{C_2H_4} = \frac{k_{diff}\left[C_2H_4\right]}{\beta}, \text{with } \phi_{C_2H_4}(t=0) = \phi_{C_2H_4,0} \quad (10)$$

Where β (72 mmol m^{-3} d^{-1} / nmol kg^{-1} s^{-1}) is a factor used to convert the measured ethylene production data in nmol kg^{-1} s^{-1} to mmol m^{-3} d^{-1}, taking into account the average density of the fruit.

3.4. Model calibration

Since no data was collected on chlorophyllase activities during ripening, the rate constants $k_{Chlase,ref}$ and $k_{Chlase,deg,ref}$, related to synthesis and degradation of Chlase respectively, were kept at some fixed values, after some initial model calibration. Also, estimated values of activation energies and associated rate constants are highly correlated, and therefore these two parameters cannot be estimated together. The activation energies for all model reactions were kept fixed at some realistic values, based on literature and some initial model fitting (see Table 1). $\left[Chlase\right]$ was normalised, such that $\left[Chlase\right]_0 = 1$. Furthermore, a^*_C was fixed at 10 (based on the highest value that was measured for apples with very yellow background colour). Therefore, the model parameters that were estimated from data were a^*_0, $f_{clim,0}$, $\phi_{C_2H_4,0}$, $k_{Chl,ref}$, $V_{max,C_2H_4,ref}$, k_{diff}, $k_{clim,ref}$, K_{m,O_2,C_2H_4}, K_{mu,CO_2,C_2H_4}, α, and γ. The model parameters were estimated by fitting the measured individual fruit data of skin chlorophyll content, background colour and ethylene production to the model Eqs. (6) – (10) using OptiPa (Hertog et al., 2007b), a dedicated optimization tool which was developed for use with Matlab (The Mathworks, Inc., Natick, MA, USA).

(Insert Table 1)

3.5. Identification of random model parameters

To account for the large fruit-to-fruit biological variability in the skin green background colour of Jonagold apples within a batch, the parameters in the model (Eqs (4) – (10)) responsible of the observed variance needed to be identified and treated as fruit specific, rather than attempting to estimate generic values for all fruit within the batch. Identification of the random factors of a kinetic model most often begins with assumptions based on prior knowledge of the system (Hertog et al., 2007c; Ochoa-Ascencio et al., 2009; Gwanpua et al., 2013). For instance in the current model, it is expected that the initial green background colour, a^*_0, which is related to how much chlorophyll was present in the skin at harvest, was the main source of variability. Also, the initial rate of ethylene production, $\phi_{C_2H_4,0}$, and the climacteric state of the fruit at harvest, $f_{clim,0}$,are both related to the maturity of the fruit at harvest (Shafiq et al., 2011; Bulens et al., 2012; Gwanpua et al., 2012), and can therefore be assumed to be a random model factor. Moreover, one can assume that all rate constants were properties of particular reactions and should be generic for all fruit, since the basic process of ripening is generic. However, as a result of some simplifications and assumptions made during the model development, the rate constants were actually lumped model parameters that were functions of other parameters related to the maturity at harvest (e.g., initial level of enzyme activities). Consequently, the model rate constants were *apparent* rate constants which varied from fruit to fruit. Finally, α and γ, which are parameters relating skin chlorophyll content to the measured colour (a^*) can be assumed to be common for all fruit.

Therefore, from a mechanistic point of view, a^*_0, $\phi_{C_2H_4,0}$, $f_{clim,0}$, $k_{Chl,ref}$, k_{diff}, $V_{max,C_2H_4,ref}$ and $k_{clim,ref}$ should all be treated as being fruit specific. Trying to obtain a separate value of all seven model parameters for each fruit would result in model over-parameterization. However, it is most likely that only a few of these model parameters were responsible for most of the observed biological variation. Several methods do exist for identifying the most significant random factors in a model, such as sensitivity analysis (Poschet et al., 2004), or by evaluating the differences in the root mean square error (RMSE)) (or the coefficient of determination) resulting from treating different model

parameters (or combinations) as being random or not (Schouten et al., 1997; Hertog et al., 2004). The RMSE is a measure of the difference between the predicted or model values and the actual data.

As a first step in identifying the most important random model parameters, a one-parameter-at-a-time sensitivity analysis was performed to identify which of the estimated model parameters do not significantly influence the predicted colour (a^*). The sensitivity of the model prediction (a^*) due to uncertainty in each model parameter was calculated using Eq. (11).

$$I_{sen,j} = \frac{a^*(p_{\max,j}) - a^*(p_{\min,j})}{a^*(p_{\text{mean},j})} \tag{11}$$

where $I_{sen,j}$ is the sensitivity index; $p_{\max,j}$, $p_{\min,j}$, $p_{\text{mean},j}$ are the upper limit, lower limit and mean values of the model parameter estimates. By using the confidence interval of the model parameter estimates, rather than some fixed perturbations, the variability introduced by the actual variations in the model parameter was taken into account. The confidence interval of the model parameters were estimated by fitting the colour model to the individual fruit data, during which separate values of all model parameters were estimated for each fruit. To calculate the sensitive of each of the model parameters, the mean, and the lower and upper limits of the estimates were inserted in Eq. (11). The model parameters with very small $I_{sen,j}$ were eliminated from the list of potentially random model parameters. To further identify which of the remaining potentially random model parameters, or combinations, were responsible for most of the observed fruit-to-fruit variability in the skin background colour, the model was re-fitted to the data, treating each of the these model parameters as being fruit specific (and later their combinations), and calculating the RMSE of a^* for the different model fits. The most important random model parameters were identified by selecting the model fit that gave the lowest RMSE (a^*), while requiring a limited number of random model parameters.

3.6. Monte Carlo simulations

The algorithm developed by Hertog et al. (2009) for the generation of correlated non-Gaussian parameters was used to generate 1000 sets of the random model parameters. 1000 Monte Carlo

simulations were carried out, using a different set of random model parameters for each run of the Monte Carlo simulations, to predict how the fruit-to-fruit variability of the skin background colour present at harvest propagates throughout storage and shelf life.

4. Results and discussion

4.1. Relationship between chlorophyll content and colour measurements

The measured chlorophyll content and the corresponding colour measurements were fitted to Eq. (9) (Fig. 1), and a correlation coefficient of 0.93 was obtained. The estimated values of α and γ, together with their standard error, are shown in Table 1.The chlorophyll content ranged from about 0.3 nmol cm^{-2} for apples with almost yellow background, to 3 nmol cm^{-2} for the very green apples. These data are similar to values reported for some different apple cultivars where skin chlorophyll content between 0.2 to 4 nmol cm^{-2} were obtained for Golden delicious and Antonovka apples (Merzlyak et al, 2003; Solovchenko et al., 2006).

(Insert Fig. 1)

4.2. General product behaviour

The a^*-value of the background colour of the fruit increased during storage and subsequent shelf life exposure as the fruit which initially had a very green background colour at harvest, turn through greenish yellow to yellow (Fig. 2). This change in colour is related to loss in skin chlorophyll pigment, causing underlying yellow pigments to be unmasked (Müller et al., 2007; Kräutler 2008). When the fruit were placed in shelf conditions, with higher temperatures, the rate of ethylene production and loss of skin greenness increased rapidly. This is because ethylene production and chlorophyll breakdown reactions are both enhanced at higher temperatures. Furthermore, the fruit stored in normal atmosphere quickly turned yellow. This was a consequence of the higher rates of ethylene production in normal air, due to the high O_2 and low CO_2 levels in normal atmosphere. It can be assumed that CA storage helped to maintain the green background colour by reducing the rate of ethylene production. A close relationship between ethylene production and the rate of loss in

greenness could be observed from the data (Fig 2). Several other authors have shown that ethylene production is closely linked to quality deterioration in climacteric fruits (Saltveit, 1999; Johnston et al., 2001; Hershkovitz et al., 2005; Gwanpua et al., 2012). From Fig. 2, ethylene production was very low throughout CA storage, due to the low O_2 levels and elevated CO_2 levels. When the fruit were placed under shelf conditions, there was a rapid increase in ethylene production, and this continued to rise until it reached a climacteric maximum, after which the ethylene production started to decrease. The climacteric maximum was easily reached by most of the fruit that had been stored in normal air.

(Insert Fig. 2)

4.3. Model calibration

Initial model calibration was done, by fitting the model to the combined colour data set, treating all model parameters as generic. This could explain only 54% of the total variance in the a^* – value of all 220 fruit during storage and shelf life. The low percentage of the explained part of the individual fruit behaviour was due to the huge biological variation which was not accounted for when attempting to obtain generic values for all model parameters. The model parameter estimates are shown in Table 1 and 2.

4.3.1. Random model parameters

The result of the sensitivity analysis for the different model parameters is shown in Fig 3. From this figure, the model predictions were very sensitive to the estimated values of a^*_0 and $k_{\mathrm{Chl},ref}$, even though this sensitivity reduced with time as the apples approached the final colour value. Also, it was deduced that during storage, the model output (a^*) was not so much influenced by changes in $\phi_{\mathrm{C_2H_4},0}$ $V_{max,\mathrm{C_2H_4},ref}$, k_{diff}, $f_{clim,0}$ and $k_{clim,ref}$. However, during shelf life, there was a climacteric rise and a rapid increase in the rate of ethylene production due to higher temperature (18 °C) and high O_2 concentration in normal atmosphere, causing the model output to become more sensitive to changes in the values of $f_{clim,0}$, and $V_{max,\mathrm{C_2H_4},ref}$. In both storage and shelf life, the model output (a^*) was not

sensitive to changes in $\phi_{C_2H_4,0}$, $k_{clim,ref}$, and k_{diff} , suggesting that even though these parameter may vary from fruit to fruit, they were not responsible for the observed fruit-to-fruit biological variation in skin background green colour. Based on the sensitivity studies, a^*_0, $f_{clim,0}$, $k_{Chl,ref}$ and $V_{max,C_2H_4,ref}$ were identified as the potentially random model parameters.

(Insert Fig. 3)

The model was re-fitted to the data of the individual fruit, separately treating each of these potentially random models parameters (and later their combinations) as being fruit specific, while keeping all other model parameters common, and calculating the RMSE (a^*) of the model fit (Fig. 4). From the result of the different model fits, a^*_0 was identified as the main source of biological variability in skin greenness, while $k_{Chl,ref}$ was also an important source of variability. On the other hand, the model parameters $V_{max,C_2H_4,ref}$, and $f_{clim,0}$, were not significant sources of variation. This suggests that although the climacteric stage of the fruit and the rate of ethylene production were important factors in determining the rate of loss of skin greenness in the apples, their variations from fruit to fruit was not sufficient to induce significant fruit-to-fruit variability in background colour within the batch. Therefore, $V_{max,C_2H_4,ref}$, $f_{clim,0}$, $\phi_{C_2H_4,0}$, k_{diff} and $k_{clim,ref}$, $k_{clim,ref}$ were assumed to be generic model parameters, while a^*_0 and $k_{Chl,ref}$ were treated as fruit-specific model parameters.

(Insert Fig. 4)

4.4. Propagation of biological variation

The mean and the standard deviations of the random model parameters, a^*_0 and $k_{Chl,ref}$, estimated from the individual fruit data is shown in Table 3. The complete dataset of the individual fruit-specific values of the random model parameters is added as supplementary material (Table 1S). It can be observed from Table 3 that different values for the mean and standard deviations of the random model parameters were obtained for fruit in the different storage conditions. This was mainly because the

355 initial distribution of the background colour of the 20 fruit in the different storage conditions were not
356 identical. A strong and positive correlation was observed between the estimated values of a^*_0 and
357 $k_{\mathrm{Chl},ref}$ (Fig. 5), in which fruit that were more mature (having higher a^*_0) had higher rate constants
358 for chlorophyll breakdown. This can be explained by considering the fact that $k_{\mathrm{Chl},ref}$ was a lumped
359 model parameter, which is most likely a function of the amount of chlorophyllase (and other
360 chlorophyll degrading enzymes) at harvest. Fruit that are beginning to ripe are already at an
361 intermediary climacteric stage, and contain significantly higher amount of enzymes, compared to fruit
362 that are less mature. This means that the more mature apples will show a higher rate of quality
363 degradation, hence a positive correlation between a^*_0 and $k_{\mathrm{Chl},ref}$. Furthermore, fruit stored under
364 normal air, and CA with elevated O_2 levels had higher mean values of $k_{\mathrm{Chl},ref}$. This suggest that in
365 addition to enhancing chlorophyllase activities through increased rate of ethylene production, elevated
366 O_2 levels during apple storage may induce other physiological processes that may accelerate the rate
367 at which chlorophyll breakdown occurs, and such details are not covered in the current model.
368 Therefore, in doing the random sampling for the Monte Carlo simulations, the random model
369 parameters were not pooled to form one big distribution. Rather, for simulations within a storage
370 condition, the distribution of the random parameters obtained for that particular storage condition was
371 used.

372 *(Insert fig. 5)*

373 Fig. 6 shows the evolution of the median, and the 50 and 95 % prediction intervals of the skin
374 background colour during storage at different conditions, followed by exposure to shelf life
375 conditions, as predicted by the Monte Carlo simulations. Also, the individual fruit measurements for
376 each sampling time, together with the calculated median, and the 50 and 95 % CI, are shown on these
377 plots. From the different plots in Fig. 6, it can be observed that the prediction intervals of the Monte
378 Carlo simulations were very closed to those calculated from the a^*- values of the individual fruit,
379 suggesting that by using Monte Carlo simulations, the model could sufficiently describe the fruit-to-

fruit variability of green background colour of 'Jonagold' apples along the postharvest chain. "The two-sample Kolmogorov Smirnov test was carried out to statistically compare the Monte Carlo predictions with the actual distributions. This was done at all measurement time points for the different CA storage conditions, and the result is shown in Table 4. From Table 4, it can observed that for the fruit stored at 1 °C and 4 °C under optimal CA for Jonagold apples (1% O_2 and 3 % CO_2), the distributions predicted by the Monte Carlo simulations are similar to the actual distributions of the a^*- values. Even for those stored under different CA conditions, the two distributions were comparable for most of the time point, except for those stored under CA with elevated CO_2 levels (10 kPa). It is possible that elevated CO_2 may influence other physiological aspects of colour breakdown not (or not completely) included in the current model.

(Insert Fig. 6)

Initially, the distribution of the background colour was slightly skewed to the left, since most of the apples had a very green background colour, with only few fruit already appearing yellow. During CA storage, the distribution changes slightly, as the fruit turn yellow, with the distribution appearing almost normal at the end of CA storage. During late shelf life, the distribution becomes skewed to the right, as most of the fruit turned yellow. Similar observations were made by Hertog et al. (2004) for the hue angle in tomatoes.

4.5. Practical applications

Knowledge on how fruit-to-fruit variability in quality within a batch propagates throughout storage is very important in managing uniformity in quality within the chain. For instance, it could help postharvest handlers to predict the probability of having apples with certain greenness after storage for some known storage conditions and duration. Such information is very important in the commercial grading of Jonagold apples, in which fruit are graded based on per cent of the apple surface with red colour, greenness of the background colour, and flesh firmness. The per cent of apple surface with red colour is a pre-harvest factor, and does not change throughout the cold chain.

405 However, the greenness of the skin background colour and the flesh firmness decreases with time
406 during storage and shelf life.

407 The model developed in the current study can help in managing variability in quality along the apple
408 cold chain, by predicting what proportion of apples in a batch will have certain level of skin
409 greenness. To illustrate this, we considered a batch of Jonagold apples with mean background colour (
410 a^*) of -8 and standard deviation of 2.5. Two scenarios of storage were considered: the normal
411 storage practice of 1 °C under CA conditions (1 % O_2 and 3 % CO_2), and 4 °C under CA conditions,
412 both for a duration of 9 months, followed by 3 weeks exposure to shelf life conditions. Three quality
413 classes based on the skin greenness were considered: high commercial value fruit with extremely
414 green background colour, having an a^*-value less than -3; fruit with greenish-yellow background
415 colour, with an intermediary commercial value, having an a^*-value between 1 and -3, and low
416 commercial value fruit with yellow background colour, having an a^*-value greater than 1. These
417 threshold values were defined based on CIE colour measurements of colour cards used for grading
418 apples at Belgian auctions.

419 By using the model, the propagation of the fruit-to-fruit variation in skin background colour of the
420 fruit in both scenarios was simulated using the Monte Carlo simulations, and the percentages of
421 apples within the different quality classes are shown in Fig. 7. About 83 % of the fruit stored under
422 the normal storage will be graded as apples with high commercial value, while 17 % will be placed in
423 the class of apples with intermediate commercial value. This suggest that although very long term
424 storage of Jonagold apples is possible under this condition, a significant proportion of the fruit cannot
425 be graded in the top quality classes. In our previous study on predicting variability in apple firmness
426 (Gwanpua et al., 2013), we predicted that more than 70 % of fruit within a batch of Jonagold apples
427 stored for 9 months, at 1°C under CA, will have a firmness greater than the critical value of 65 N,
428 required for high quality graded apples. In the other scenario shown in Fig. 7, in which the fruit were
429 stored at 4 °C under CA, it can be seen that 35 % of the apples will fall in the class with intermediate
430 commercial value, because of the higher rate of colour degradation. Conversely, the maximal time
431 allowed to store the apples at 4 °C under CA, while ensuring the fruit retain the same quality level as

those under normal storage conditions for 9 months, was calculated, and the probability distribution of the background colour at the end of storage in that scenario is shown in Fig. 7. This maximal time at 4 °C turns out to be five months. The implication of this is that if postharvest handlers are able to predict when the fruit will be taken out of storage, then storage could be done at slightly higher temperatures, thereby reducing electricity cost. Again, we used the model of Gwanpua et al. (2013) to predict what percentage of the apple will have firmness greater than the critical firmness for high quality graded apples, and we obtained 85 %. This suggest that although the flesh firmness is an extremely important quality indicator for Jonagold apples, proper management of the skin greenness will always result in fruit with acceptable flesh firmness. Nevertheless, other quality aspects (such as core browning) need to be considered before such dynamic energy saving strategy could be employed.

(Insert fig. 7 and 8)

As a second example of the practical applications of the model developed in this study for predicting variability in skin background colour, the batch keeping quality of apples at the retailer was predicted for Jonagold apples that are typically displayed under shelf conditions (18 °C) and for apples that are kept in a walk-in cold room at 12 °C. Batch keeping quality was defined as the time it takes for 10 % of the apples to reach a background colour limit. The colour limit was set at an a^*-value of 2, obtained based on the relationship between the CIE colour measurements and colour card values used by the Belgian retailers for assessing quality of apples. The percentage of apples with a^* value greater than 2 was simulated during shelf life exposure at 12°C and 18°C for both apples that had been stored for 9 months at 1 °C under CA, and for 5 months at 4°C under CA (Fig. 8). It can be seen that apples stored at 12 °C in walk-in cold rooms can be kept three times longer than those stored under ambient temperature. However, if it is expected that all the fruit are sold within two weeks, then based on the model, keeping at low temperatures may not be necessary. Also, based on the simulation, there is no difference in the evolution of skin background colour for both apples stored for 9 months at 1 °C under CA and for 5 months at 4 °C under CA during shelf life exposure at 12 °C.

Conclusions

458 Biological variability in quality for postharvest produces can be better managed if postharvest
459 handlers are able to predict how the biological variation present at harvest propagates throughout the
460 chain. Stochastic modelling offers a unique tool for managing biological variability. The model
461 developed in this study covers most of the important mechanisms associated with loss in the green
462 background colour in apples, and can easily be extended to conditions different, but similar, to those
463 under which the model was developed. It provides a useful tool that can be used in proper
464 management of uniformity in quality within a batch of apples.

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585

Table 1. Mean model parameter estimates, together with the standard deviations (S.D.) for the part of the model describing ethylene production.

[(1)]**Parameter**	[(2)]**Estimate (S.D.)**	**Units**
$\phi_{C_2H_4,0}$	0.003 (0.053)	nmol kg^{-1} s^{-1}
$V_{max,C_2H_4,ref}$	5490 (1070)	mmol m^{-3} d^{-1}
E_{a,C_2H_4}	111400	J
$f_{clim,0}$	0.0003 (0.0002)	-
$k_{clim,ref}$	0.15 (0.014)	-
$E_{a,clim}$	150000	J
K_{m,O_2,C_2H_4}	6.5 (1.7)	kPa
K_{mu,CO_2,C_2H_4}	41 (94)	kPa
k_{diff}	0.0069 (0.0011)	d^{-1}

[(1)] $\phi_{C_2H_4,0}$ is the initial rate of ethylene production; $f_{clim,0}$ is the initial climacteric stage; $V_{max,C_2H_4,ref}$ (mmol m^{-3} d^{-1}) is the maximum rate of ethylene production, at a reference temperature of 10°C; K_{m,O_2,C_2H_4} (kPa) and K_{mu,CO_2,C_2H_4} (kPa) are the Michaelis-Menten constants for ethylene production and uncompetitive inhibition of ethylene production by carbon dioxide respectively; E_{a,C_2H_4} and $E_{a,clim}$ are the activation energies for ethylene production and rate of change in climacteric stage of the fruit, and k_{diff} is a rate constant relating ethylene diffusion from the tissue to the surrounding.

[(2)] Parameters with no standard deviations were kept fixed during model calibration.

Table 2. Mean model parameter estimates, together with the standard deviations (S.D.) for the part of the model describing loss of green background colour.

[(1)]**Parameter**	[(2)]**Estimate (S.D.)**	**Units**
a^*_0	-7.7 (2.5)	-
$k_{\mathrm{Chl},ref}$	0.018 (0.026)	$d^{-1}\ m^{-3}\ mmol$
$k_{\mathrm{Chlase},ref}$	0.0000039	$d^{-1}\ m^{3}\ mmol^{-1}$
$k_{\mathrm{Chlase},deg,ref}$	0.000057	d^{-1}
$E_{a,\mathrm{Chl}}$	150000	J
$E_{a,\mathrm{Chlase}}$	100000	J
$E_{a,\mathrm{Chlase,\ deg}}$	80000	J
α	13.6 (0.3)	-
γ	0.38 (0.03)	-
a^*_C	10	-

[(1)] a^*_0 is the initial a^*- value of the skin background; α and γ are constants relating chlorophyll content to colour (a^*); $k_{\mathrm{Chl},ref}$, $k_{\mathrm{Chlase},ref}$, and $k_{\mathrm{Chlase},deg,ref}$ are the rate constants for chlorophyll breakdown, synthesis of chlorophyllase, and breakdown of chlorophyllase respectively (all rate constants are reported at a reference temperature of 10°C), and $E_{a,\mathrm{Chl}}$, $E_{a,\mathrm{Chlase}}$, and $E_{a,\mathrm{Chlase,\ deg}}$ are the activation energies for chlorophyll breakdown, synthesis of chlorophyllase, and turnover of chlorophyllase respectively.

[(2)] Parameters with no standard deviations were kept fixed during model calibration.

Table 3. Mean and standard deviation (S.D) of the estimated values of the stochastic model parameters ($k_{Chl,ref}$ and a^*_0) obtained by fitting the model (Eqs. (4) – (10)) to the individual fruit data, for Jonagold apples stored under different conditions.

Storage condition [(1)] (T-O_2-CO_2)		**No storage**	**1-1-3**	**1-1-10**	**1-3-3**	**1-3-10**	**1°C NA[(2)]**	**4-1-3**	**4-1-10**	**4-3-3**	**4-3-10**	**4°C NA**
$k_{Chl,ref}$ (d^{-1} m^{-3} mmol)	Mean	0.0091	0.0135	0.0091	0.0158	0.0125	0.0180	0.0120	0.0111	0.0199	0.0127	0.0440
	S.D	0.0018	0.0037	0.0017	0.0050	0.0019	0.0071	0.0032	0.0017	0.0067	0.0029	0.0193
a^*_0	Mean	7.5	6.3	8.5	7.6	8.1	5.9	8.2	10.2	7.5	9.5	5.3
	S.D	2.3	2.5	1.7	2.5	1.8	1.7	2.4	2.0	2.0	1.6	2.3

[(1)] T is the temperature in °C, O_2 and CO_2 are the oxygen and carbon dioxide levels in kPa. After storage, the apples were placed in shelf life conditions (1 °C, 20.8 % O_2 and 0.03 % CO_2) for 15 days.
[(2)] NA is storage under normal atmosphere.

Table 4. *p*-values of the two-sampled Kolmogorov Smirnov test between the Monte Carlo simulations and the actual fruit measurements.

Storage condition [(1)] (T-O_2-CO_2)	**[(2)]Measurement point (d)**						
	0	60	127	170	175	180	185
1 -1- 3	0.889	0.853	0.712	0.589	0.349	0.310	0.507
1- 1 -10	0.392	0.159	0.135	0.278	0.025*	0.020*	0.000*
1 -3- 3	0.771	0.929	0.581	0.536	0.392	0.975	0.013*
1- 3 -10	0.771	0.779	0.727	0.719	0.022*	0.091	0.000*
4 -1 -3	0.650	0.612	0.604	0.589	0.398	0.143	0.000*
4- 1 -10	0.000*	0.223	0.150	0.064	0.001*	0.000*	0.000*
4 -3- 3	0.019*	0.219	0.337	0.044*	0.214	0.135	0.002
4- 3 -10	0.042*	0.764	0.507	0.099	0.087	0.008*	0.000*

[(1)] T is the temperature in °C, O_2 and CO_2 are the oxygen and carbon dioxide levels in kPa. After storage, the apples were placed in shelf life conditions (1 °C, 20.8 % O_2 and 0.03 % CO_2) for 15 days.

[(2)] Measurement points 175, 180 and 185 represent measurements done when the fruit were placed under shelf conditions.

* Distribution of the Monte Carlo simulations was statistically different, at a 5 % significance level, from the distribution of the 20 fruit measurements.

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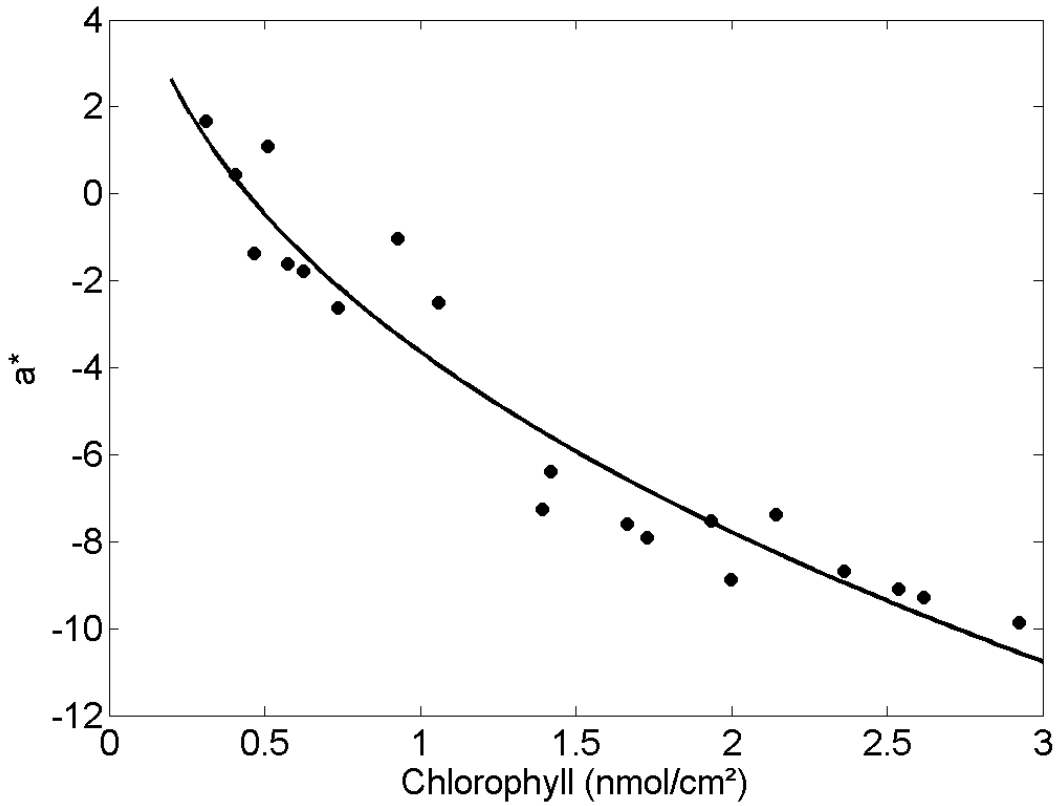

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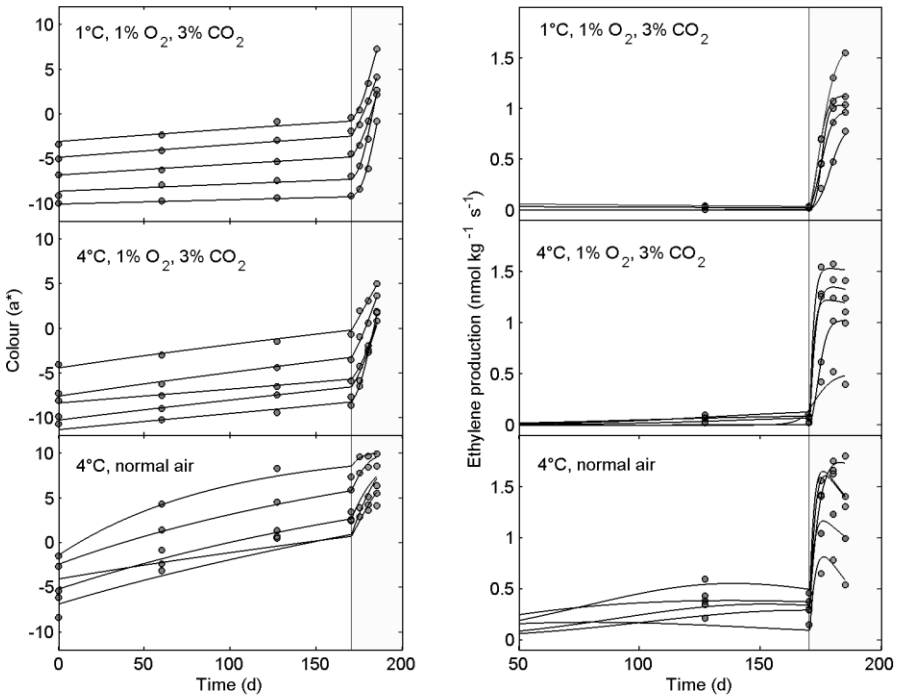
637 Figure 1



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639 Figure 2

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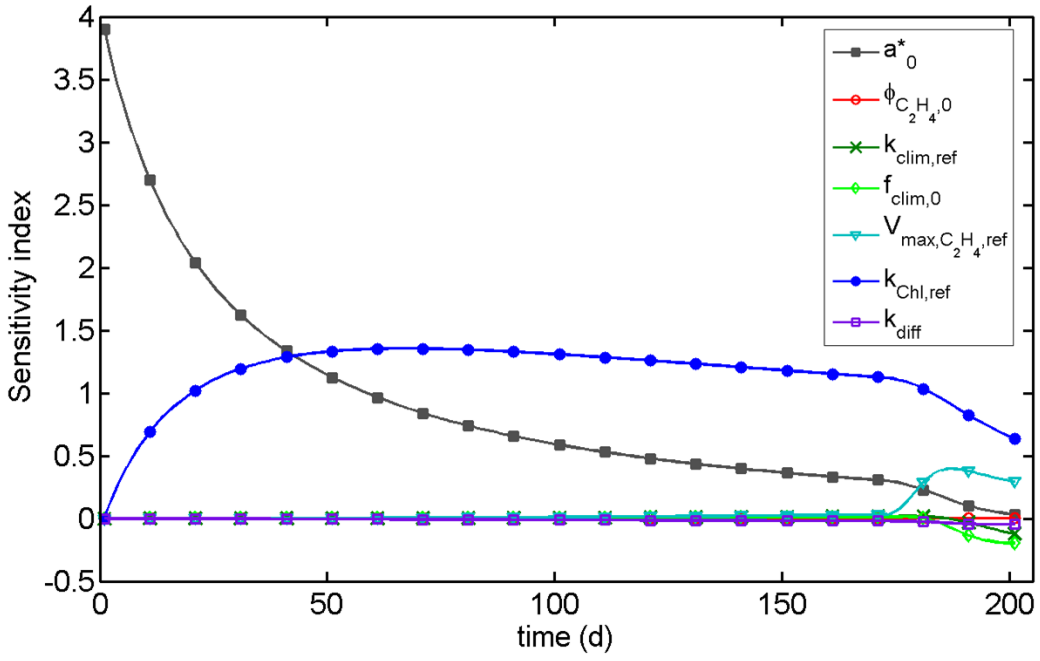

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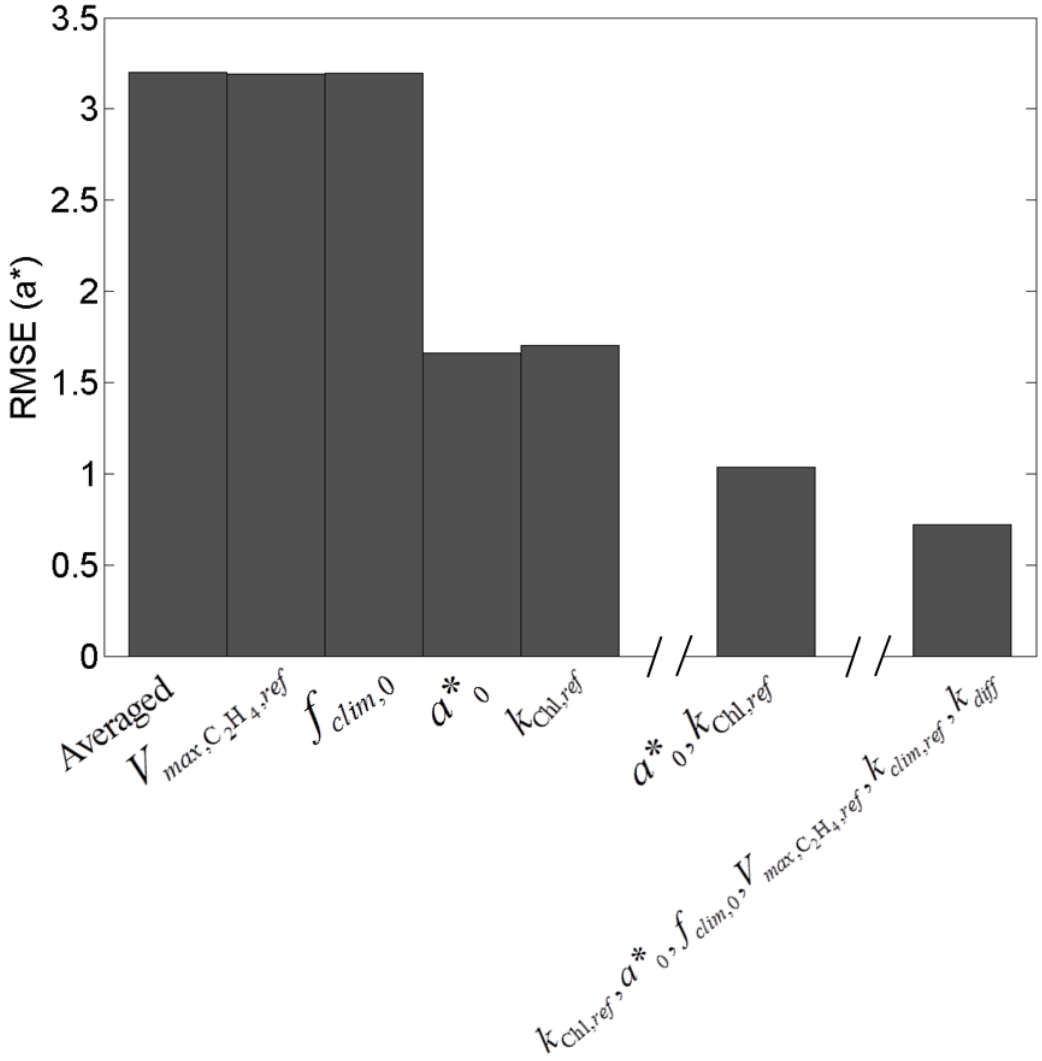
642 Figure 3



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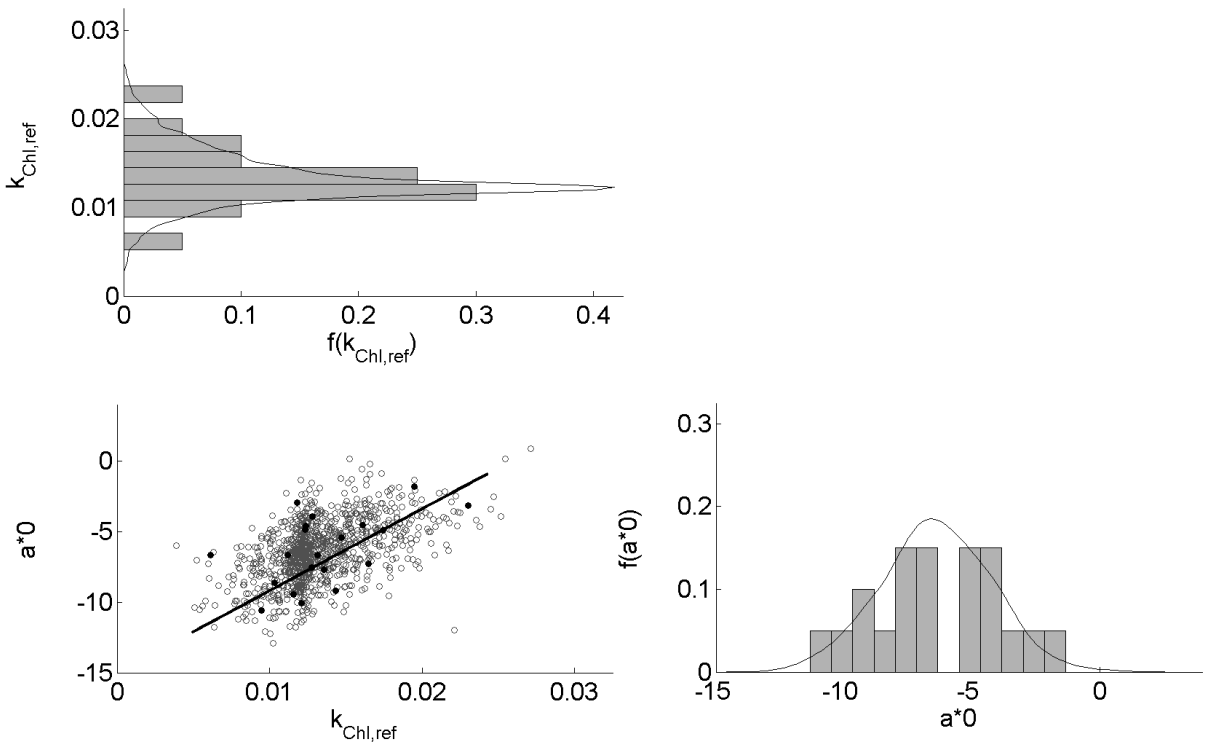
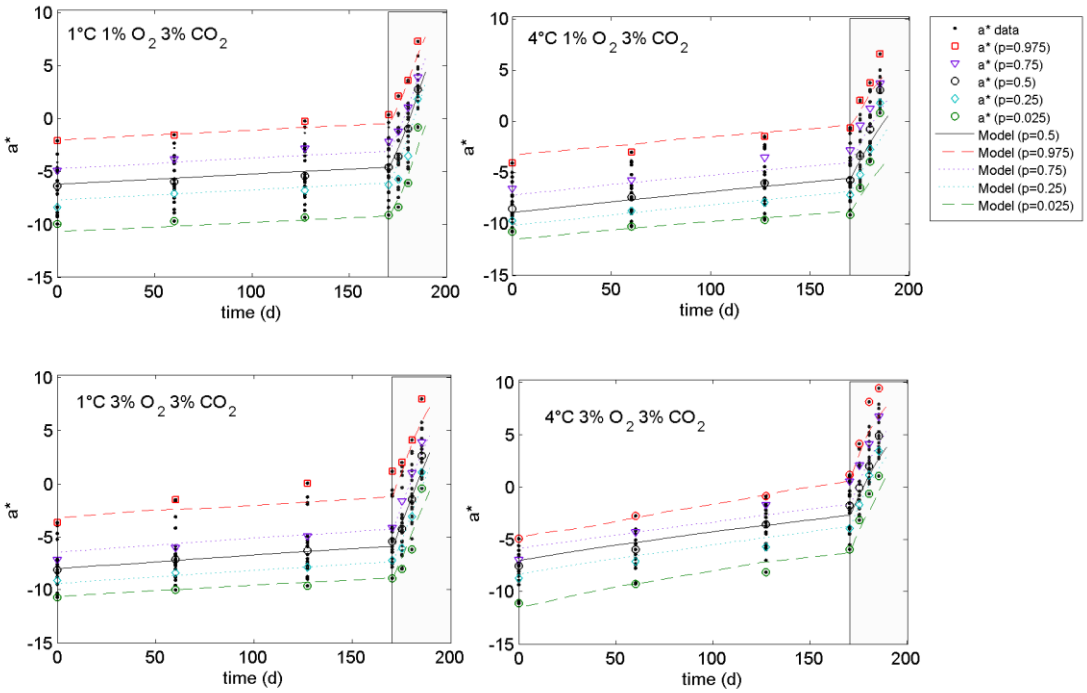
644 Figure 4



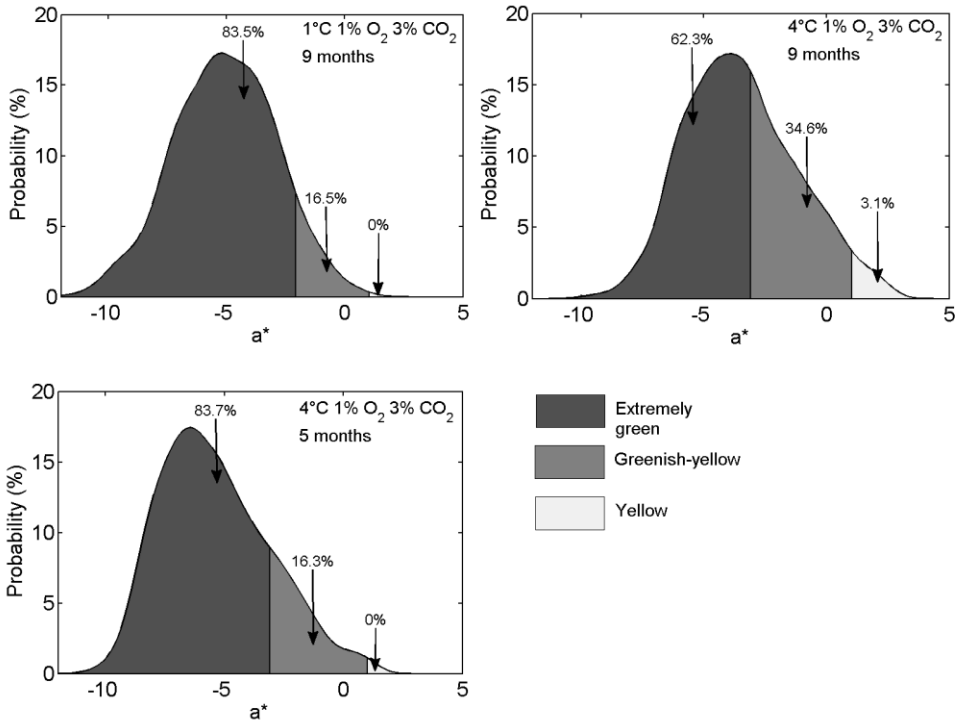
645

646 Figure 5



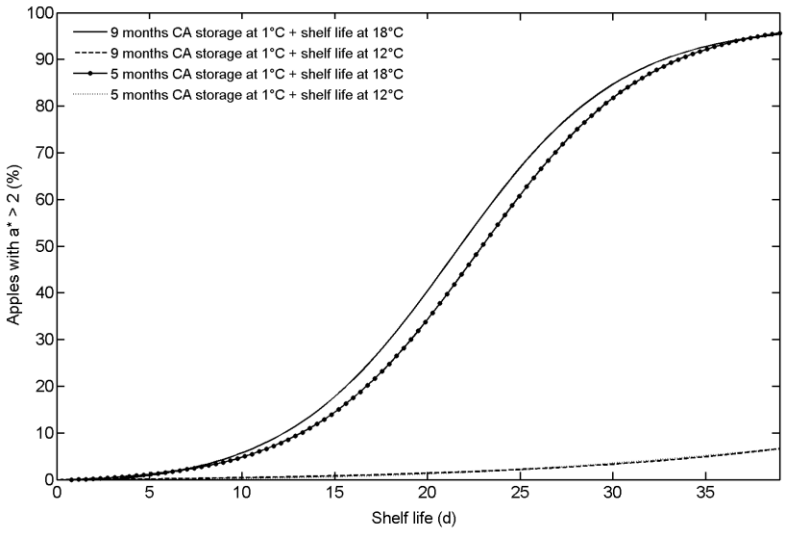
647

648 Figure 6



649

650 Figure 7



651

652 Figure 8

653

Figure captions

Fig. 1. Relationship between chlorophyll content and green background colour (a^*-value) of Jonagold apples, as described by Eq. (9). The line is the model, while the points are the measurements.

Fig. 2. Evolution of skin background colour (a^*) and ethylene production of five randomly selected individual apples during storage of Jonagold apples at different conditions, followed by exposure to shelf life conditions from 170 days on (shaded portion). The lines are the modelled values (using fruit specific model parameters), while the points are the measurements.

Fig. 3. Sensitivity analysis of predicted a^*-value of skin background colour with respect to the different model parameters, $\phi_{C_2H_4,0}$ (the initial rate of ethylene production), $k_{Chl,ref}$ (the rate constant for the breakdown of chlorophyll), $V_{max,C_2H_4,ref}$ (the rate constant for ethylene production) , $k_{clim,ref}$ (the rate constant for the rate of change in climacteric stage of the fruit), k_{diff} (rate constant for ethylene diffusion from inside the fruit to its surrounding), $f_{clim,0}$ (climacteric state of the fruit at harvest) and a^*_0 (initial a^*-value of the skin background colour) .

Fig. 4. The root mean square error (RMSE (a^*)) of different model fits, compared to the model describing the averaged batch behaviour, and the model fit in which all parameters were allowed to be fruit-specific. The labels on the horizontal axes indicate which model parameter(s) were estimated for each of the individual fruit. a^*_0 is the initial a^*-value of the skin background colour; $k_{Chl,ref}$ is the rate constant for the breakdown of chlorophyll; $k_{clim,ref}$ is the rate constant for the rate of change in climacteric stage of the fruit; $f_{clim,0}$ is the initial climacteric state of the fruit, $\phi_{C_2H_4,0}$ is the initial rate of ethylene production, $V_{max,C_2H_4,ref}$ is the rate constant for ethylene production, and k_{diff} is the rate constant for ethylene diffusion from the fruit to its surrounding.

676 Fig 5. Frequency distribution and the correlation matrix of the random model parameters for fruit
677 stored at 1°C under controlled atmosphere conditions (1% O_2 and 3% CO_2). $k_{Chl,ref}$ (d^{-1} m^{-3} mmol) is
678 the rate constant for the breakdown of chlorophyll and a^*_0 is the initial skin background colour)

679 Fig. 6. Simulations of the propagation of the fruit-to-fruit variability in skin background colour (a^*)
680 during storage at different conditions, followed by exposure to shelf life conditions (grey region).

681 Fig. 7. Simulations of the percentage of fruit with extremely green, greenish-yellow and yellow
682 background colour for fruit stored at 1°C and 4°C under controlled atmosphere (CA) for 9 months,
683 and for fruit stored at 4°C under CA for 5 months.

684 Fig. 8. The percentage of fruit with background colour beyond consumer acceptance ($a^* > 2$), as a
685 function of shelf life duration, at 12°C and 18°C.

686

687

Highlights

- A model linking ethylene to loss of green background colour of apples was developed
- Relation between skin chlorophyll content and CIE colour (a*) was established
- Monte Carlo method was used to model fruit-to-fruit variability in background colour
- Batch keeping quality was predicted for different storage and shelf life conditions

694 Supplementary Table S1

Fruit number	Storage condition	$k_{Chl,ref}$	a^*_0
1	No storage	0.030903	8.91648
2		0.049888	8.93613
3		0.036052	4.44275
4		0.041527	11.8388
5		0.054734	9.17013
6		0.03691	9.93272
7		0.047455	6.55916
8		0.034862	5.90206
9		0.039531	9.2002
10		0.022625	10.139
11		0.029624	6.99018
12		0.037278	6.39329
13		0.038343	2.02808
14		0.045688	7.6401
15		0.031446	7.85451
16		0.03668	9.77101
17		0.041587	5.6449
18		0.040986	8.15223
19		0.035171	8.83511
20		0.03879	10.2147
21	1°C ; 1% O_2; 3% CO_2	0.062588	3.93354
22		0.038192	7.4396
23		0.036997	5.59081
24		0.039512	8.53061
25		0.049945	5.76254
26		0.040254	9.98102
27		0.04594	8.08149
28		0.036754	8.24977
29		0.017823	7.00792
30		0.04644	5.35037
31		0.041798	6.10878
32		0.032785	10.1037
33		0.036174	5.24353
34		0.029486	9.18909
35		0.033593	10.7296
36		0.02692	11.1615
37		0.03861	4.67101
38		0.055045	2.56662
39		0.036784	3.66084
40		0.031935	7.23683
41	1°C ; 1% O_2; 10%	0.033871	9.90511

	CO_2		
42		0.027372	10.6898
43		0.024383	10.5918
44		0.025231	10.7717
45		0.03307	10.0589
46		0.027645	8.70617
47		0.031624	8.93779
48		0.033725	7.68648
49		nan	nan
50		0.024667	6.55139
51		0.02875	8.58446
52		0.028469	10.9206
53		0.032186	5.82606
54		0.022218	10.0096
55		0.036675	6.82611
56		0.019085	10.444
57		0.028555	8.16108
58		0.025812	9.24371
59		0.024632	5.92327
60		0.040683	7.11084
61	1°C ; 3% O_2; 3% CO_2	0.025463	9.474
62		0.034846	8.43605
63		0.024934	10.1158
64		0.041102	8.87817
65		0.057273	4.12016
66		0.025172	11.7911
67		0.030933	11.4538
68		0.035644	8.87542
69		0.032406	3.69353
70		0.02229	11.7369
71		0.027662	9.43025
72		0.03123	9.61776
73		0.046359	5.17954
74		0.028575	11.6341
75		0.029024	8.8221
76		0.047106	5.96793
77		0.035822	10.9982
78		0.050302	9.71531
79		0.029666	8.43597
80		0.028293	8.44263
81	1°C ; 3% O_2; 10% CO_2	0.027901	9.84343
82		0.037474	11.054
83		0.034172	10.5053
84		0.03108	11.1738
85		0.037081	9.24357

86		0.036684	9.0017
87		0.027921	9.35583
88		0.028412	6.38344
89		0.034721	10.442
90		0.027693	5.2371
91		0.04053	8.1307
92		0.038657	9.92473
93		0.033921	4.20587
94		0.031148	10.6108
95		0.036664	8.8807
96		0.039944	8.28886
97		0.0313	8.11924
98		0.040193	8.24019
99		0.046471	8.1837
100		0.036798	9.43427
101	1°C ; Normal air	0.024572	8.20332
102		0.025692	8.50421
103		0.021316	7.37164
104		0.023958	5.71827
105		0.017218	9.16091
106		0.026091	8.83158
107		0.0266	6.84216
108		0.017392	10.4189
109		0.017943	11.5434
110		0.02927	10.2801
111		0.016937	9.38056
112		0.025246	9.07064
113		0.03283	7.7761
114		0.024525	11.0747
115		0.015907	10.8432
116		0.043206	7.13138
117		0.028977	6.98902
118		0.027599	9.58027
119		0.02669	9.3773
120		0.036578	10.7687
121	4°C ; 1% O_2; 3% CO_2	0.032797	10.8529
122		0.027906	8.78326
123		0.030008	11.8923
124		0.03442	9.74084
125		0.043368	5.99774
126		0.031962	11.6745
127		0.037944	4.7319
128		0.041425	7.74032
129		0.045795	4.35269
130		0.040795	9.61265
131		0.05997	5.99827

132		0.040212	9.6358
133		0.034178	12.2333
134		0.02797	10.1276
135		0.038606	8.00504
136		0.013723	10.0225
137		0.020109	5.54565
138		0.04069	8.17791
139		0.03485	8.48845
140		0.033643	10.4977
141	4°C ; 1% O_2; 10% CO_2	0.037028	11.1023
142		0.034512	10.3788
143		0.036057	10.4706
144		0.04643	9.72096
145		0.026543	11.6929
146		0.038007	11.3279
147		0.03627	12.483
148		0.041524	4.27345
149		0.046333	11.1111
150		0.041557	11.0518
151		0.033227	11.1766
152		0.039744	7.06368
153		0.041343	9.0134
154		0.024934	12.1765
155		0.034911	9.47947
156		0.03222	11.7543
157		0.042356	8.59454
158		0.035394	11.7464
159		0.036338	11.242
160		0.03882	10.8084
161	4°C ; 3% O_2; 3% CO_2	0.026367	9.39537
162		0.034525	9.79492
163		0.054042	7.25654
164		0.042743	12.697
165		0.031015	9.82558
166		0.058838	8.4792
167		0.03224	7.0285
168		0.031182	7.34545
169		0.038696	8.30199
170		0.038696	10.7397
171		0.038321	7.4595
172		0.053423	5.9021
173		0.033247	8.41431
174		0.048796	7.46034
175		0.042851	6.06547
176		0.032921	8.11571

177		0.040199	9.90694
178		0.041414	12.8815
179		0.064856	9.5367
180		0.042247	6.44462
181	4°C ; 3% O_2; 10% CO_2	0.031587	11.9556
182		0.023116	12.1319
183		0.030722	11.5992
184		0.050134	9.08404
185		0.039659	6.18075
186		0.033344	10.6709
187		0.042356	9.9678
188		0.04341	8.83788
189		0.0413	11.0971
190		0.028375	10.696
191		0.035328	8.23008
192		0.042783	8.60937
193		0.053508	11.2509
194		0.042896	8.00708
195		0.029505	9.23769
196		0.032193	11.5323
197		0.029448	10.2526
198		0.033759	9.52419
199		0.032411	10.8266
200		0.030978	10.7552
201	4°C ; normal air	0.031218	7.63808
202		0.022468	5.43569
203		0.042451	7.94575
204		0.035764	8.28091
205		0.026146	7.28824
206		0.04469	6.54488
207		0.039342	7.22198
208		0.03825	8.3787
209		0.029847	8.22774
210		0.03194	5.30278
211		0.041877	10.6109
212		0.047464	7.34981
213		0.044084	2.95936
214		0.030122	5.87437
215		0.026443	9.71745
216		0.035776	6.46769
217		0.029833	3.66124
218		0.071957	1.56765
219		0.037865	9.8342